

- 2 -

**Amendments to the Specification:**

Please replace the paragraph of the substitute specification filed 12/04/2005 between lines 5-20 on p. 17 with the following amended paragraph:

Cloning and expression vectors suitable for the needs of the practitioner can be selected from various, commercially available vectors that are compatible with prokaryotic hosts, *e.g.*, pBR322, pUC, pET, and which also contain marker sequences conferring antibiotic resistance. The foregoing systems are particularly compatible with *E. coli*. Other prokaryotic hosts, *e.g.*, strains of *Bacillus* and *Pseudomonas*, can be used with compatible control sequences known to those of ordinary skill in the art. In specific embodiments, described *infra*, the vector pET28b(+) (Novagen, Madison, Wisconsin) is used to express HCV NS3 helicase fragments. It is noted that due to a subcloning artifact from pET28b(+), these constructs have a G-S-H-M polypeptide sequence (residues 1-4 of SEQ ID NO:4) at the amino-terminus. Numerous expression control sequences are available for prokaryotes, including promoters, optionally containing operator portions, and ribosome binding sites, *e.g.*, T7 bacteriophage promoter [Dunn and Studier, *J Mol Biol*, **166**:477 (1983)],  $\beta$ -lactamase (penicillinase) and lactose promoter systems [Chang *et al.*, *Nature*, **198**:1056 (1977)], tryptophan (*trp*) promoter system [Goeddel *et al.*, *Nuc Acids Res*, **8**:4057 (1980)],  $\lambda$ -derived  $P_L$  promoter and N gene ribosome binding site [Shimatake *et al.*, *Nature*, **292**:128 (1981)] and hybrid tac promoter [De Boer *et al.*, *Proc Nat Acad Sci USA*, **292**:128 (1983)].

Please replace the paragraph of the substitute specification filed 12/04/2005 beginning on line 21 on p. 17 and ending on line 7 of p. 18 with the following amended paragraph:

Eukaryotic hosts can be used as desired, including without limitation, yeast (*e.g.*, *Saccharomyces*, *Klebsiella*, *Picia*, and the like) and mammalian cells in culture systems. Yeast-compatible vectors and control sequences are well known in the art and can carry markers that permit selection of successful transformants by conferring prototrophy to auxotrophic mutants or resistance to heavy metals on wild-type strains. Mammalian cell lines available as hosts for expression are known in the art and include many immortalized cell lines available from the

86343\_1.DOC

- 3 -

American Type Culture Collection® (ATCC®) organization, including HeLa cells, Chinese hamster ovary (CHO) cells, baby hamster kidney (BHK) cells, and a number of other cell lines. Suitable promoters for mammalian cells are also known in the art, and include viral promoters from, *e.g.*, Simian Virus 40 (SV40) [Fiers *et al.*, *Nature*, **273**:113 (1978)], Rous sarcoma virus (RSV), adenovirus (ADV), and bovine papilloma virus (BPV), glyceraldehyde-3 phosphate dehydrogenase (GAPDH) promoter or alcohol dehydrogenase (ADH) regulatable promoter, terminators also derived from GAPDH, and if secretion is desired, a leader sequence derived from yeast  $\alpha$ -factor (*see* U.S. Pat. No. 4,870,008). Mammalian cells may also require or benefit from terminator sequences, *e.g.*, derived from the enolase gene [Holland, *J Biol Chem*, **256**:1385 (1981)], and poly-A addition sequences, enhancer sequences (which increase expression), sequences which promote gene amplification, *e.g.*, methotrexate resistance genes, which are known in the art.

Please replace the paragraph of the substitute specification filed 12/04/2005 between lines 14-24 on p. 18 with the following amended paragraph:

After expression of an HCV NS3 helicase fragment, HCV NS3 helicase polypeptide fragments can be isolated and purified according to conventional methods in the art, typically depending upon the type of expression system used. In specific embodiments, illustrated *infra*, HCV NS3 helicase fragments are expressed from pET28b(+) in *E. coli* and isolated by lysing cells and centrifuging to obtain the supernatant which contains the HCV NS3 helicase fragment. The supernatant is subjected to Ni<sup>2+</sup> chelation chromatography to purify the fragment, which binds to the column due to the presence of an amino-terminal His tag on the fragment. The isolated fragment is then proteolytically cleaved with thrombin to remove the histidine tag. These fragments have a four residue sequence G-S-H-M (residues 1-4 of SEQ ID NO:4) at the amino terminus, which does not effect the function of the fragments. After thrombin proteolysis, the fragment of interest are separated from the histidine tag, *e.g.*, by size exclusion chromatography.